COLORADO RIVER RECOVERY PROGRAM Project No.: C18/19
FY-2006 -2009 PROPOSED SCOPE OF WORK for:
Chemically Fingerprinting Nonnative Fishes in Reservoirs

Lead Agency: Colorado Division of Wildlife
Submitted by:

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Date: January 11, 2006
Revised: February 28, 2006, per Biology Committee (2/28/06 by Pat Nelson)

Category:
___ Ongoing Project
_X_ Ongoing-revised project
___ Requested new project
___ Unsolicited proposal

Expected Funding Source:
_X_ Annual funds
___ Capital funds
___ Other (explain)

I. Title of Proposal: Chemically Fingerprinting Nonnative Fishes in Reservoirs

II. Relationship to RIPRAP:

This proposal addresses movement of nonnative fish into river reaches of critical habitat from reservoirs known to support cool- and warmwater species of nonnative fish. These species include northern pike, smallmouth bass, largemouth bass, black crappie, and walleye. These species are believed to pose a significant predatory threat to the young life stages of endangered and other native fishes (Tyus and Saunders 1996; Martinez et al. 2001; Johnson et al. 2005a). However, it is uncertain to what extent the presence of nonnative species in critical habitat is the result of escapement or illicit transfers from reservoirs. Overall, this study is intended to develop chemical fingerprints of nonnative fishes in 11 reservoirs that are potential sources of nonnative fishes to the critical habitat of Upper Colorado River Basin through microchemical analysis of otoliths. Successful development of these fingerprints will provide the means to assess the proportion of nonnative fishes in these rivers that originate from reservoirs and thereby guide management efforts to reduce this influx of nonnative fishes.

III. Study Background/Rationale and Hypotheses:

Background/Rationale:

Nonnative fishes are present throughout the Upper Basin (Martinez 2002, Trammel et al. 2002), and can adversely impact the recovery of endangered fishes through predation or competition at critical life stages or in critical locales. However, the recruitment sources and origins of nonnative fishes are not well known. Immigration of nonnative fishes from nearby reservoirs has been demonstrated in some cases by the recapture of fishes that had been tagged as part of other studies. However, large scale tagging efforts to address the growing concern about escapement of nonnative piscivores from multiple reservoirs throughout the Upper Basin is impractical. This Scope of Work seeks to verify fish escapement from reservoirs as a source of nonnative fish entering critical habitat by applying newly developed techniques for identifying naturally occurring markers via microchemical analysis of otoliths.

Otolith microchemistry provides a means to trace the origins and movements of fishes in marine (Humpreys et al. 2005, Campana et al. 2000; Bath et al. 2000) and freshwater environments (Brazner et al. 2004, Bronte et al. Wells et al. 2003). In freshwater systems differences in underlying geology can result in water chemistry that varies among watersheds. Limnological processes and chemical transformations within reservoirs impart further distinctiveness to water chemistry among lentic and lotic water bodies. Chemical composition of ambient water is imparted to otoliths of resident fish in a highly predictable and temporally referenced manner. Because otoliths are physiologically inert structures their chemical composition does not change after material is accreted. Thus, otoliths record the environmental history of a fish and that information can be used to determine the fish's provenance (origin and movements).

Recent work by Whitledge et al. (in review; in prep.) has demonstrated that otolith microchemistry has excellent potential for tracing the provenance of nonnative fishes in the Upper Colorado River Basin. Further, graduate work by CSU students Ryan Fitzpatrick and Daniel Gibson-Reinemer is showing that many water bodies (ponds, streams, reservoirs) and hatcheries in Colorado possess unique chemical fingerprints, and that these fingerprints are imparted to the otoliths of fish originating from each location. It also appears that transfers of fish can be detected in otoliths as shifts in the chemical composition along laser transects performed with laser ablation inductively coupled plasma mass spectrometry (LA-ICP-MS; Johnson et al. 2005b). These findings coupled with the highly heterogeneous nature of the Colorado Plateau's geology suggest that otolith microchemistry is likely to reveal new insights into the movements of nonnative fishes within the Upper Colorado River Basin.

Hypotheses:

We hypothesize that:

a. the chemical composition (fingerprints) of otoliths from nonnative fishes will differ among reservoirs,
b. inter-annual variation in otolith fingerprints will be small relative to inter-reservoir differences,
c. otolith core signatures of fishes that were reared in reservoirs and immigrated to rivers in critical habitat will be distinct from signatures of fishes inhabiting rivers since hatching, and
d. otolith core signatures can be used to identify fishes as having originated from a particular reservoir.

IV. Study Goals, Objectives, End Product:

Study Goals: to determine chemical "fingerprints" of nonnative fishes in reservoirs that are potential sources of nonnative fishes to critical habitat.

Study Objectives:

Primary objectives of the investigation will be to:

1. quantify chemical "fingerprints" of fishes within study reservoirs and evaluate the degree of inter-annual variation in those fingerprints.
2. determine if fish sampled in rivers the vicinity of study reservoir possess otolith core signatures that identify them as having originated from one of the study reservoirs.
3. improve our understanding of the degree to which immigration or transfers from reservoirs contributes to the load of nonnative fishes in critical habitat of the Upper Colorado River basin.
4. provide recommendations to guide management efforts to reduce the influx of nonnative fishes from reservoirs.

End Products:

1. A quantitative tool to determine the proportion of nonnative fishes in critical habitat that originate from reservoirs.
2. A forensic tool to assist conservation officers in prosecuting individuals engaged in the illegal transfer of nonnative fishes from reservoirs.
3. Identification of the origin and contributing sources of target nonnative fishes to critical habitat, to facilitate the fiscal and ecological efficiency of nonnative fish control.

V. Study Area:

The principal area of study for this SOW will be large reservoirs within the Upper Colorado River Basin, including those in northeastern Utah, southwestern Wyoming and western Colorado (Bottle Hollow, Crawford, Flaming Gorge, Harvey Gap, Kenney, McPhee, Paonia, Ridgeway, Rifle Gap, Rio Blanco and Starvation reservoirs).

VI. Study Methods/Approach:

Nonnative fishes will be collected by standard fisheries sampling techniques, collateral to ongoing sampling by state, federal or university efforts. The number of species varies by reservoir and river, but will include northern pike, smallmouth bass, largemouth bass, black crappie and walleye. We will extract sagittal otoliths from up to 20 individuals of each species from each site. Otoliths will be removed from fishes using non-metallic forceps, rinsed with distilled water, and stored dry in polyethylene vials until preparation for analyses. A range of fish sizes/ages will be collected to allow us to examine otolith core (first year of life) signatures across a number of year classes, and thereby assess inter-annual variation in those signatures. We will strive to make two collections per year from seven reservoirs in 2007 and 2008, and one per year in the remaining four. Water samples will be collected for microchemical analysis at the time of fish sampling.

Otoliths will be embedded in Epo-fix® epoxy, sectioned in a transverse plane using an ISOMET low-speed saw, and polished to reveal annuli. Otolith thin sections will be mounted on acid-washed glass slides using double-sided tape, ultrasonically cleaned for 5 min in ultrapure water, and dried for 24 h under a laminar flow hood. We will employ well-established methods for the microchemical analysis using LA-ICP-MS (Campana 1999) in addition to new techniques developed with Recovery Program funding by Whitledge et al. (in review).

Dr. Brett Johnson of the Department of Fishery and Wildlife Biology at CSU will hire and supervise a graduate research associate (M.S.) to identify sampling intensity, conduct and oversee microchemical analyses, evaluate data and provide findings. CDOW will maintain oversight of this project and will assist and coordinate field sample collection in close cooperation with the graduate research associate. Analytical work will be conducted under the guidance of Alan Koenig, U.S.G.S. Research Scientist, using the LA-ICP-MS instrument at the U.S.G.S. Mineral Resources Laboratory in Denver, Colorado.

This study will compliment recent work that estimated the degree of immigration of nonnative fishes to the Colorado River from floodplain ponds and backwaters (Martinez and Martinez 2004, Whitledge et al. in review, Whitledge et al.in prep).

VII. Task Description and Schedule:

<u>FY 2006</u>:

Task 1. Field Collections.

Pat Martinez, CDOW Aquatic Researcher and field technicians will lead field collection efforts in cooperation with the graduate research associate. Preliminary reservoir sampling will be conducted during June-September 2006. This sampling will be coordinated with the respective states and crews operating in the target reservoirs and river reaches.

Task 2. Microchemical Analysis of Otoliths.
Dr. Brett Johnson at CSU will recruit a graduate research associate, and as soon as funding for FY07 is confirmed he will select a graduate research associate to perform analyses and interpretation of otolith samples and assist with field collections.

FY 2007:
Task 1. Field Collections.
Pat Martinez, CDOW Aquatic Researcher and field technicians will lead field collection efforts in cooperation with the graduate research associate. Full scale reservoir and river sampling will be conducted during May through August 2007, with two collections made at seven reservoirs and one in the remaining four. This sampling will be coordinated with the respective states and crews operating in the target reservoirs and river reaches.

Task 2. Microchemical Analysis of Otoliths.
The graduate student will begin in January 2007. Work will involve year round sample and data analysis, the graduate research associate will advise CDOW on field sample collection and ongoing sample preparation for microchemical analyses. The graduate research associate will submit quarterly reports to Pat Martinez, CDOW.

Reporting: An annual report will be submitted to Pat Nelson by December 15, 2006.

FY 2008:

Task 1. Pat Martinez, CDOW Aquatic Researcher and field technicians will lead field collection efforts in cooperation with the graduate research associate. Reservoir and river sampling will be conducted during May through August, with two collections made at seven reservoirs and one in the remaining four.

Task 2. CSU graduate research associate will perform analyses and interpretation of otolith samples. The graduate research associate will submit quarterly reports to Pat Martinez, CDOW.

Reporting: An annual report will be submitted to Pat Nelson by December 15, 2007. Graduate research associate will present preliminary findings at the Upper Basin Researcher's Meeting in January 2008.

FY 2009:

Task 1. No activity unless findings in previous years warrant additional sampling.

Task 2. CSU graduate research associate will perform analyses and interpretation of otolith samples. The graduate research associate will submit quarterly reports to Pat Martinez, CDOW. The graduate research associate will submit M.S. thesis to graduate committee in April 2009.

Reporting: Graduate research associate will present preliminary findings at Upper Basin Researcher's Meeting in January 2009.
Draft final report to Pat Nelson – May 15, 2009.
1st revised draft final report to peer review – June 15, 2009. (peer reviews due to author – July 15, 2009; BC comments due – August 3, 2005).
2nd revised draft final report to Biology Committee – September 3, 2009

Project Timeline:

<table>
<tr><th>Month</th><th>2006</th><th>2007</th><th>2008</th><th>2009</th></tr>
<tr><td>Jan</td><td></td><td>Grad student begins</td><td>Upper Basin presentation</td><td>Upper Basin presentation</td></tr>
<tr><td>Feb</td><td></td><td rowspan="3">Prepare and analyze otoliths (LA-ICP-MS)</td><td rowspan="3">Prepare and analyze otoliths (LA-ICP-MS)</td><td rowspan="2">Data analysis and writing</td></tr>
<tr><td>Mar</td><td></td></tr>
<tr><td>Apr</td><td></td><td>Thesis draft</td></tr>
<tr><td>May</td><td></td><td rowspan="3">Field collections; Prepare and analyze otoliths (LA-ICP-MS)</td><td rowspan="3">Field collections; Prepare and analyze otoliths (LA-ICP-MS)</td><td>Draft final report</td></tr>
<tr><td>Jun</td><td rowspan="3">Field collections; Prepare otoliths for LA-ICP-MS; recruit grad student</td><td>Revised final report, draft 1</td></tr>
<tr><td>Jul</td><td></td></tr>
<tr><td>Aug</td><td></td><td rowspan="2">Data analysis and report writing; Prepare and analyze otoliths (LA-ICP-MS)</td><td rowspan="2">Data analysis and report writing; Prepare and analyze otoliths (LA-ICP-MS)</td><td></td></tr>
<tr><td>Sep</td><td>Revised final report, draft 2</td></tr>
<tr><td>Oct</td><td>Recruit grad student</td><td rowspan="2"></td><td rowspan="2"></td></tr>
<tr><td>Nov</td><td>Data analysis and report writing;</td><td></td></tr>
<tr><td>Dec</td><td>Interim report</td><td>Interim report</td><td>Interim report</td><td></td></tr>
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VIII. FY- 2006 through 2009 Work:

FY 2006 Deliverables:

Summary of field collections provided in annual report to Program- December 2006.

FY 2007 Deliverables:

Presentation of preliminary findings at Upper Basin Researcher's Meeting (January 2007). Preliminary findings summarized in annual report to Program- December 2007.

FY 2008 Deliverables:

Presentation of preliminary findings at Upper Basin Researcher's Meeting (January 2008). Findings summarized in annual report to Program- December 2008.

FY 2009 Deliverables:

Draft Final Report distributed for peer-review by 30 May 2009.

Budget

FY 2006 Costs:

Task 1 - Field Collection	
Supplies	$500
Travel, vehicle	$4,000
University indirect cost @ 15% (funds passed through existing Larval Lab- or BMR-USBR agreement)	$675
Total	$5175
Task 2 - Otolith Analysis	
CSU professor salary, fringe (0.25 months)	$2,392
Student hourly & fringe (800 hours)	$9,984
Travel	$500
Lab supplies	$500
University indirect cost @ 15% (funds passed through existing Larval Lab- or BMR-USBR agreement)	$2006
Total	$15382
TOTAL (FY 2006)	**$20,557**

FY 2007 Costs:

Task 1 - Field Collection	
Supplies	500
Travel, vehicle	2,000
University indirect cost @ 15% (funds passed through existing Larval Lab- or BMR-USBR agreement)	375
Total	$2,875
Task 2 - Otolith Analysis	
Graduate Research Associate & fringe	12,636
Graduate tuition (1 semester)	1,751
CSU professor salary, fringe (1 month)	9,000
Student hourly & fringe (300 hours)	3,744
Mass spectrometer use fees	5,333
Water analysis fees	1,500
Travel	500
Lab supplies	500
University indirect cost @ 15% (funds passed through existing Larval Lab- or BMR-USBR agreement)	4,982
Total	$39,946
TOTAL (FY 2007)	**$42,821**

FY 2008 Costs:

Task 1 - Field Collection	
Supplies	0
Travel, vehicle	0
University indirect cost @ 15% (funds passed through existing Larval Lab- or BMR-USBR agreement)	0
Total	0
Task 2 - Otolith Analysis	
Graduate Research Associate & fringe	$13712
Graduate tuition (1 semester)	$1,839
CSU professor salary, fringe (1.125 months)	11302
Student hourly & fringe (200 hours)	$2,496
Mass spectrometer use fees	$2,800
Water analysis fees	$1,000
Travel	525
Lab supplies	400
University indirect cost @ 15% (funds passed through existing Larval Lab- or BMR-USBR agreement)	4835
Total	$38,909
TOTAL (FY 2008)	**$38,909**

FY 2009 Costs:

Task 1 - Field Collection	
Total	$0
Task 2 - Otolith Analysis	
Graduate Research Associate & fringe	7,020
Graduate tuition (1 semester)	1,930
CSU professor salary, fringe (0.56 months)	7,097
Mass spectrometer use fees	0
Water analysis fees	0
Travel	551
Lab supplies	400
Journal page charges	500
University indirect cost @ 15% (funds passed through existing Larval Lab- or BMR-USBR agreement)	2,335
Total	$19,834
TOTAL (FY 2009)	**$19,834**

IX. Budget Summary:

FY-2006	
Field Collection:	$ 5,175
Otolith Analyses:	$15,382
Total	**$20,557**
FY-2007	
Field Collection:	$ 2,875
Otolith Analyses:	$39,946
Total	**$42,821**
FY-2008	
Field Collection:	$ 0
Otolith Analyses:	$38,909
Total	**$38,909**
FY-2009	
Field Collection:	$ 0
Otolith Analyses:	$19,834
Total	**$19,834**
Amount Requested from Recovery Program (FY07 – FY09)	**$122,121**

X. Reviewers: Anita Martinez, Colorado Division of Wildlife

XI. References:

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